Volume 248, number 1,2, 119–122 FEB 07119 May 1989

A barley flour inhibitor of insect α-amylase is a major allergen associated with baker's asthma disease

Domingo Barber, Rosa Sánchez-Monge*, Luis Gómez*, José Carpizo, Alicia Armentia+, Carlos López-Otín°, Fernando Juan and Gabriel Salcedo*

*Departamento de Análisis, Alergia e Inmunología Abelló, *Departamento de Bioquímica, ETS Ingenieros Agrónomos, Madrid, +Sección de Alergia, Hospital Rio Ortega, Valladolid* and *°Departamento de Bioquímica, Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain*

Received 7 March 1989

A barley salt-soluble protein of 14.5 kDa, which inhibits the α-amylase from the insect *Tenebrio molitor*, has been identified as a major IgE-binding component of sera from baker's asthma patients. The N-terminal amino acid sequence of this protein indicates that it is a member of a previously described family of α-amylase/trypsin inhibitors.

Amylase inhibitor, α-; Allergen; Baker's asthma; (Barley)

1. INTRODUCTION

Baker's asthma, an allergy to inhalation of cereal flours, is a widespread disease affecting a considerable number of people involved in flour manipulation [1,2]. Although this has been known for many years, little progress has been made in the identification of major flour allergens [3]. The most prominent components involved in flour allergy are salt-soluble proteins [4,5], and a number of allergens included in this protein class have been detected in wheat, however, their purification has not been reported [3,5]. A substantial fraction of the salt-soluble proteins of wheat and barley endosperms is represented by a single protein family of α-amylase/trypsin inhibitors [6], in which a clear homology has been reported between the corresponding components from both species [6,7].

Correspondence address: G. Salcedo, Departamento de Bioquímica, ETS Ingenieros Agrónomos, 28040 Madrid, Spain

Abbreviations: IEF, isoelectrofocusing; RAST, radioallergosorbent test; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SGE, starch gel electrophoresis; RP-HPLC, reversed-phase high-performance liquid chromatography

The fact that bread wheat is an allohexaploid (genomes AABBDD), with potentially triplicate sets of genes encoding homologous products, further hinders the study of their allergenic proteins. It has been shown that sera from subjects sensitized to wheat flour react significantly with seed extracts from barley [8], which is closely related to wheat and has a less complex mixture of genetic variants due to its diploid nature. We report here the identification of a member of the barley α-amylase/trypsin inhibitor family as a major allergen associated with baker's asthma disease.

2. MATERIALS AND METHODS

Salt-soluble proteins from endosperms (*Hordeum vulgare* cv. Bomi) were obtained as described [9]. Preparative RP-HPLC of the 150 mM NaCl, extract, precipitated with 50% saturated $(NH_4)_2SO_4$, was performed on a Vydac C4 column (22 × 250 mm, particle size 10 μm) eluted with a two-step linear gradient (20–50%) of acetronitrile in 0.1% trifluoroacetic acid (linear 20–35% in 150 min, linear 35–50% in 100 min; 2 ml/min). Mini-slab SDS-PAGE [10] and two-dimensional gel electrophoresis [9] were carried out as described. N-terminal amino acid sequencing of C-carboxymethylated allergen was performed by automated Edman degradation using an Applied Biosystems model 477A sequencer; the resulting phenylthiohydantoin amino acid derivatives were identified using a model

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

120 A on-line PTH analyzer and the standard Applied Biosystems program. α-Amylase inhibitor tests were performed as in [11] and included 0.1 mM $CaCl_2$ in the buffers.

A pool of seven sera from patients with baker's asthma was used in immunoblotting and RAST inhibition experiments. The pool was RAST class 4 when assayed with wheat or barley flour disks from Pharmacia. Anti-human IgE-Mab Hamlet®-^{125}I (Algeria e Inmunologia Abello) was used as detection reagent in both types of experiments. Immunoblotting of proteins from SDS-PAGE gels was carried out according to Lughtenberg et al. [12] and RAST inhibition essentially as in [13].

3. RESULTS AND DISCUSSION

A salt-soluble protein preparation, obtained from barley flour by extraction with 0.15 M NaCl and precipitation with 50% $(NH_4)_2SO_4$, was fractionated by reversed-phase HPLC (fig.1A). 14 fractions were collected as indicated (fig.1A). Appropriate aliquots from these fractions were subjected to SDS-PAGE and either stained with Coomassie blue (fig.1B) or immunoblotted (fig.1C). Most of the allergenic activity was associated with two protein bands of apparent molecular mass 27 and 14.5 kDa, respectively (fig.1C). The 27 kDa allergen did not appear to be eluted from the column, whereas practically all the 14.5 kDa IgE-fixing capacity was associated with a single peak, which upon re-chromatography yielded a single component, as judged by SDS-PAGE and two-dimensional electrophoresis (fig.2).

Two independent RAST-inhibition experiments were carried out, using a 0.15 M NaCl extract from barley endosperm, coupled to cellulose, and an ap-

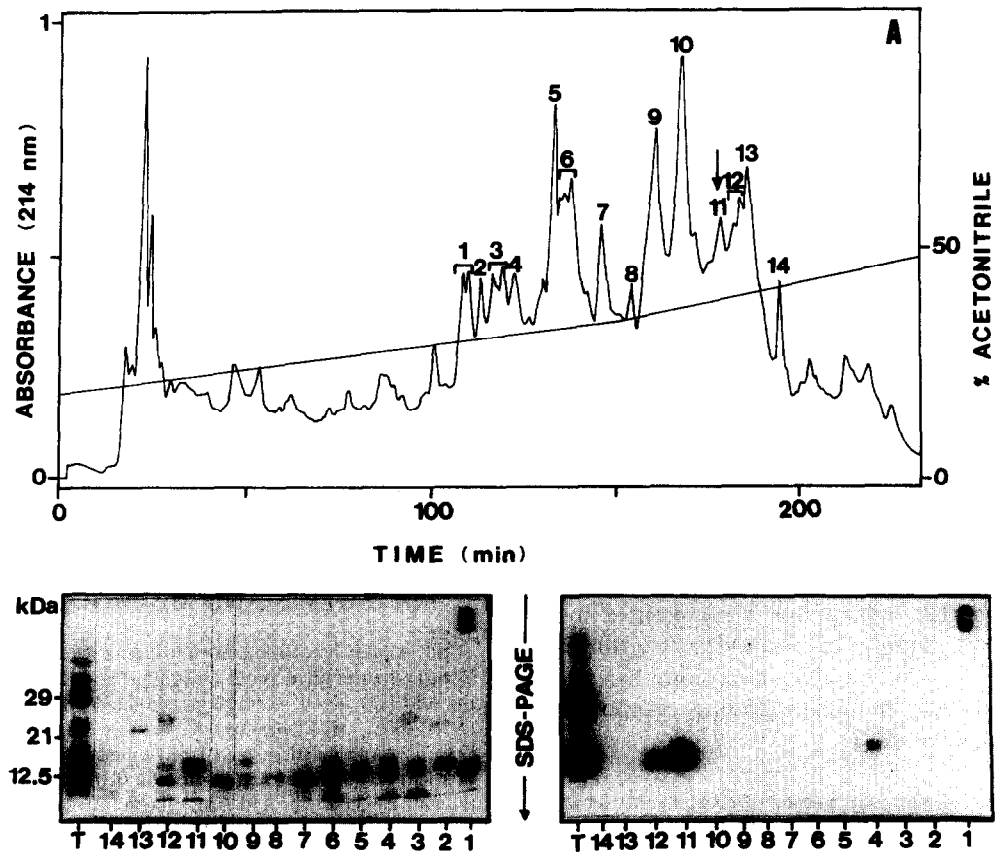

Fig.1. (A) HPLC fractionation on Vydac-C4 of the 0.15 M NaCl extract from barley flour precipitated with 50% saturated $(NH_4)_2SO_4$. Fractions further analyzed by SDS-PAGE and immunoblotting are indicated by numbers. The peak corresponding to the 14.5 kDa allergen is denoted by an arrow. (B) SDS-PAGE of the following samples: (T) 0.15 M NaCl extract precipitated with $(NH_4)_2SO_4$; (1-14) HPLC fractions as indicated in A. (C) Immunoblot of a replica of the gel in B probed with pooled sera from baker's asthma patients and radiolabelled anti-IgE.

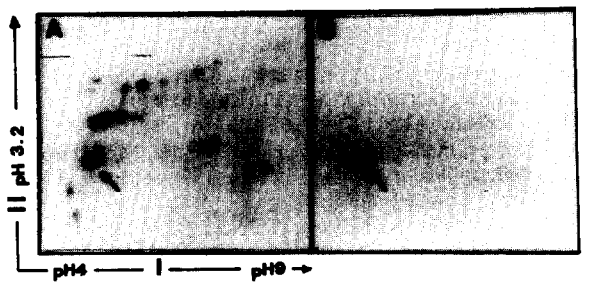

Fig.2. Two-dimensional electrophoresis (electrofocusing, pH 4–9 × starch-gel electrophoresis, pH 3.2) of the following samples: (A) 0.15 M Nacl extract precipitated with $(NH_4)_2SO_4$; (B) purified 14.5 kDa barley allergen.

propriate dilution of the pooled sera. The pure allergenic protein, at a concentration of 10 μg/ml, caused inhibition which was 40 ± 10% of that caused by a 1 mg/ml solution of the total extract. This result is in agreement with the semi-quantitative estimation that can be inferred from the immunoblot of the total extract in fig.1C.

The N-terminal amino acid sequence of the C-carboxymethylated protein was determined. A single sequence was obtained, confirming that the allergen was a single protein. The sequence clearly indicated homology of this protein with monomeric and dimeric α-amylase inhibitors from barley and wheat (fig.3) and appeared to be identical to that described as S15 by Barber et al. [14]. Whether wheat flour contains proteins with a closer sequence relationship to the allergen reported here than those aligned in fig.3 remains to be investigated.

To characterize the possible inhibitory properties of the protein, inhibition tests were carried out with

Table 1

Inhibitory activity of the 14.5 kDa barley allergen against α-amylases from the insect *Tenebrio molitor* and from human saliva

Inhibitor	Amount of protein (μg)	Inhibition of α-amylase (%)[a]	
		Tenebrio	Salivary
14.5 kDa allergen	1	70	0
	5	85	0
BDAI-1	1	21	0
	5	38	0
0.28	1	100	27
	5	100	64
0.19	1	51	78
	5	76	95

[a] 1 unit of enzyme, defined as the amount of the enzyme that produces the reducing equivalents of 1 μmol maltose in 30 min, was used per assay

The barley dimeric inhibitor, BDAI-1, and the wheat monomeric (0.28) and dimeric (0.19) inhibitors were used for comparison

α-amylases from the insect *Tenebrio molitor* and from human saliva (table 1). The protein was an effective inhibitor of the insect enzyme at concentrations that had no effect on that of human.

In investigation of the stability of this allergen during the processing of foods that include barley products, e.g. beers, soups, etc., is warranted in connection with prevention of its adverse effects.

Comparison of the amino acid sequence of the allergen, when completed, with those of the other members of the same protein family which are not allergenic should be of great help in the identification of the critical epitopes.

A study of wheat allergens that would correspond to the 14.5 kDa barley species is underway.

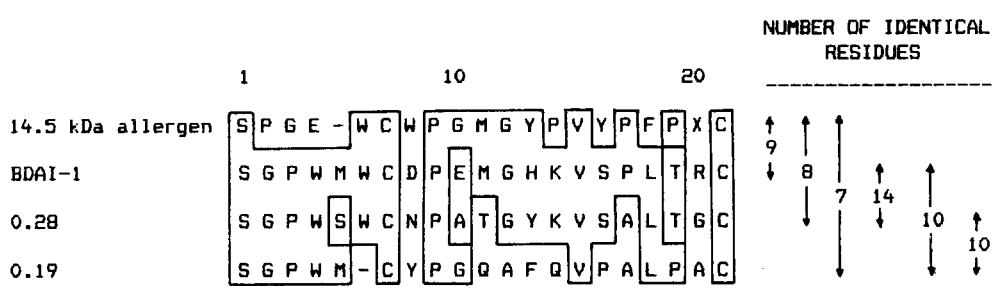

Fig.3. Alignment of the N-terminal sequence of the 14.5 kDa barley allergen with those of the barley dimeric inhibitor BDAI-1 [15] and the wheat monomeric, 0.28 [16], and dimeric, 0.19 [17], inhibitors. Numbers of identical residues for all binary comparisons are listed on the right.

Acknowledgements: We thank Dr F. García-Olmedo for his advice and help in preparing the manuscript, and J. García-Guijarro and D. Lamoneda for technical assistance. This work has been supported in part by grant no. PB85-0193 from Comisión Interministerial de Ciencia y Tecnología.

REFERENCES

[1] Thiel, H. and Kallweit, C. (1984) Allergologie 7, 407–414.
[2] Thiel, H. and Ulmer, W.T. (1980) Chest 78, 4005–5055.
[3] Baldo, B.A. and Wrigley, C.W. (1984) Adv. Cereal Sci. Technol. 6, 289–356.
[4] Baldo, B.A. and Wrigley, C.W. (1978) Clin. Allergy 8, 109–124.
[5] Theobold, K. (1986) J. Allergy Clin. Immunol. 78, 470–477.
[6] García-Olmedo, F., Salcedo, G., Sanchez-Monge, R., Gomez, L., Royo, J. and Carbonero, P. (1987) Oxford Surveys Plant Mol. Cell Biol. 4, 275–334.
[7] Barber, D., Sanchez-Monge, R., García-Olmedo, F., Salcedo, G. and Mendez, E. (1986) Biochim. Biophys. Acta 873, 147–151.
[8] Baldo, B.A., Krilis, S. and Wrigley, C.W. (1980) Allergy 35, 45–56.
[9] Sanchez-Monge, R., Gomez, L., García-Olmedo, F. and Salcedo, G. (1986) FEBS Lett. 207, 105–109.
[10] Laemmli, U.K. (1970) Nature 227, 681–685.
[11] Barber, D., Sanchez-Monge, R., Mendez, E., Lazaro, A., García-Olmedo, F. and Salcedo, G. (1986) Biochim. Biophys. Acta 869, 115–118.
[12] Lughtenberg, B., Meijers, J., Peters, R., Van der Hoek, P. and Van Alphen, I. (1975) FEBS Lett. 58, 254–258.
[13] Ceska, M., Eriksson, R. and Varga, E. (1972) J. Allergy Clin. Immunol. 49, 1–9.
[14] Barber, D., Limas, G.G., Gavilanes, J.G. and Mendez, E. (1988) Planta 176, 221–229.
[15] Lazaro, A., Sanchez-Monge, R., Salcedo, G., Paz-Ares, J., Carbonero, P. and García-Olmedo, F. (1988) Eur. J. Biochem. 172, 129–134.
[16] Kashlan, N. and Richardson, M. (1981) Phytochemistry 20, 1781–1784.
[17] Maeda, K., Kakabayashi, S. and Matsubara, H. (1985) Biochim. Biophys. Acta 828, 213–221.